California Department of Food and Agriculture
Science Advisory Panel on *Phytophthora ramorum* in the Nursery Industry

Gary Chastagner, Doug Cook, Robert Linderman, James MacDonald, Brett Tyler

September 1-2, 2004 Meeting Summary

During the September 1 public forum, representatives of the California Department of Food and Agriculture provided an overview of Sudden Oak Death and *Phytophthora ramorum* issues in California. Nick Condos presented a historical overview of Sudden Oak Death from its first observation and discovery as a new disease in wildland areas, to the subsequent discovery of *P. ramorum* on nursery stock and the resultant regulatory actions. Courtney Albrecht then presented a summary of the national survey results for California, describing additional nursery finds and the regulatory actions they triggered. Finally, Cheryl Blomquist described the sampling and diagnostic methods used to detect and verify the presence of *P. ramorum* in plants, with particular emphasis on nursery stock.

Following these presentations, Kathy Kosta of the CDFA presented a series of questions that the Advisory Panel was asked to consider. Participants in the public session were allowed to ask additional questions, or to sharpen the focus of questions. The panel then retired to a closed session to deliberate their responses. Below are the questions that were considered by the panel, and the corresponding responses. These responses were presented in a public forum on September 2.

Question 1. Based on the research to date, what is the known pest risk of *Phytophthora ramorum* and sudden oak death?

Response: This was described as a rhetorical question, so the response was couched in general terms stating some known facts. (1) *Phytophthora ramorum* is an introduced (exotic) pathogen; (2) *P. ramorum* is known to cause disease on an expanding list of plant species (the exact number being unknown); (3) In the nursery environment, *P. ramorum* causes diseases that can be managed readily with fungicides that currently are used to control other *Phytophthora* diseases; (4) Outside the nursery environment, some hosts are only slightly affected by *P. ramorum* (e.g., minor leafspots) and some are severely affected (i.e., killed); and (5) Limiting identifiable pathways of pathogen spread is a reasonable means of slowing disease spread (the testing of seed lots to prevent movement of seed-borne diseases, or the cutting down of citrus trees to prevent spread of citrus canker were cited as examples).

The panel noted that, given the right host and conditions, *P. ramorum* has the potential to cause serious disease. And while there are many *Phytophthora* species that nursery growers must defend against already, *P. ramorum* is distinguished from many of these species by its ability to sporulate prolifically on certain hosts, and its dehiscent sporangia that allow rapid, aerial spread of the pathogen. Most other species of *Phytophthora*, some of which are commonly encountered in nurseries as root pathogens, do not have airborne dispersal phases, and thus do not have the same spread potential within susceptible crops.

It also should be noted that preliminary results from two sources indicate that *P. ramorum* may infect roots, and that studies by Linderman indicate that *P. ramorum* can survive several months in some potting mixes. If confirmed, these findings would indicate additional avenues of pathogen spread via container-grown plants (apart from the known avenues associated with foliar infections).

Research needs: The issues of host range, sporulation potential on different hosts, and the efficiency of inoculum dispersal in the nursery environment need additional research in order to fully characterize factors contributing to transmission risk.

Question 2. Can you characterize the risk of moving the pathogen and/or disease from one location to another via the movement of nursery stock? Do different host species or cultivars present different risks?

Response: Clearly there already has been confirmed movement of the pathogen within the nursery industry on infected plant material. This has been clearly demonstrated in European countries and their nurseries. The most profound example of this to date in the U.S., occurred in the spring of 2004 when host plants (camellias) from a nursery in southern California were shipped to many locations throughout the United States. A subsequent disease outbreak in the southern California nursery triggered trace-forward searches for all host material from that nursery. The trace-forwards led to positive detections and the destruction of host plants at numerous locations across the country. Thus, movement of the pathogen with host plants is a verified risk. The panel noted that introduction of *P. ramorum* into a new area does, in fact, present a risk (albeit impossible to accurately quantify) that nearby susceptible plants will become infected. Conditions influencing such spread are not yet fully characterized or quantified, but the reality of that risk is amply illustrated by the presence of Sudden Oak Death in California. The genetic evidence shows that *P. ramorum* was introduced into California and, while the method of introduction is unknown, it was likely a single introduction.

The panel also noted that different host species and cultivars likely present different risks with regard to spread, due to differences in susceptibility or sporulation potential. *Phytophthora ramorum* sporulates profusely on the leaves of some plants, and more sparsely on the leaves of others. There could be similar differences in chlamydospore formation in infected tissues. Thus, in addition to active leaf infections, old infected, abscised plant leaves or other debris within the plant canopy or littering the surface of containers may represent another means of pathogen dispersal pathogen within and between nurseries. Furthermore, the potting medium itself may become infested with propagules if sporangia or infected tissues drop to the surface of containers and become incorporated into the media. Movement of the pathogen in recycled water within a nursery as well as in natural or landscape situations should also be considered.

Research needs: As stated above, we need to know more about relative susceptibility and sporulation potential on different hosts, the efficiency of inoculum dispersal and host plant infection under nursery or landscape conditions, and the production and survival of propagules in plant debris.

Question 3. What information does the scientific literature provide regarding disease transmission? What conditions are necessary for disease development?

Response: The scientific literature and reports of research in progress, all indicate that the most common mechanism of transmission is airborne splash dispersal. Splash dispersal of inoculum is consistent with other aerial *Phytophthora* diseases that occur in nursery, landscape, agricultural, and natural settings (e.g., *Phytophthora palmivora*), and there is some indication that *P. ramorum* may splash from soil onto plants in natural settings. While reasonable inferences can be drawn from the literature based upon the behavior of other species, the precise time/temperature/humidity relationships for formation, dispersal, and infectivity of *P. ramorum* propagules has not yet been described in sufficient detail to allow prediction of conditions that favor activity by this particular species.

Research needs: We need a greater understanding of the environmental conditions and cultural practices that can influence disease incidence, as well as the physiological state of the host.

Question 4. What criteria does the panel believe should be used to determine the host status of a particular plant species or cultivar?

Response: The Panel recognizes Koch's postulates (i.e., recovery of a microbe from an infected host, isolation of the microbe in pure culture, inoculation of a healthy host with a pure isolate of the pathogen to reproduce the disease, and finally, re-recovery of the pathogen from the inoculated host) as the accepted criteria for establishing the pathogenicity of an organism to a given host. Experimental inoculations of a proven host with other isolates of the same pathogenic organism can be used to subsequently determine susceptibility to a range of isolates. Similarly, experimental inoculations also can provide useful information about the potential susceptibility of additional plant cultivars or species to a given pathogen. However, because experimental inoculations may not precisely replicate the inoculum forms, nutrient status, or inoculum levels encountered in nature, or the environmental conditions associated with natural infections, caution must be exercised in interpreting the results of such experiments and their significance with regard to the true host range of a pathogen.

The Panel noted that a variety of approaches have been used to implicate plants as hosts or associated hosts, with regard to *P. ramorum*. Following are examples of different testing methods which have been used as evidence:

1. Symptoms occur in nature and Koch's Postulates satisfied.
2. Symptoms occur in nature; *P. ramorum* detected by PCR but not cultured. Susceptibility established using an alternate isolate.
3. Symptoms occur in nature, *P. ramorum* recovered in culture, re-inoculation step of Koch's Postulates not completed.
4. Symptoms not observed in nature, but plant susceptible when inoculated with an isolate of *P. ramorum*.
5. *P. ramorum*-like symptoms occur in nature; positive PCR; but *P. ramorum* not cultured and symptoms not reproduced when plant challenged with a known isolate.

The panel noted that different plants can exhibit a range of responses, ranging from highly susceptible to completely immune following inoculation with a pathogen. Differences in inoculation methodologies, differences in the testing methods used to establish an implication (examples listed above), and small numbers of plants are all factors that can interact to confound test interpretations. Thus, the panel noted that any tests used to determine host status and define host range should be subjected to peer review.

The panel also pointed out that the susceptibility of one plant species does not necessarily implicate other species within the same genus or even other cultivars within a species. Indeed, using other *Phytophthora* diseases as examples, it is common to encounter differences in susceptibility ranging from susceptible to resistant, among cultivars of the same plant species.

Question 5. Has any work been done, or is there any information in the literature, that indicates that the host list for the A1 and the A2 strains are the same?

Response: The Panel noted that recent research indicates that the European (A1) isolates appear to be more aggressive on susceptible plants than North American (A2) isolates. However, research also has shown considerable variation in aggressiveness among North American isolates and most of the studies to date have utilized only a limited number of isolates. Apart from possible differences in aggressiveness on known hosts, the limited data presently available suggests that differences in host range between A1 and A2 strains may be minimal.

Research need: More work is needed to clearly establish any differences in host range and aggressiveness, and to better understand the consequences should the A1 strains become established in North America. *(In progress, see citation.)*

Question 6. Is there information showing that potting soil or water will transmit the disease under natural conditions?

Response: In Europe, there are reports that *P. ramorum* has been isolated from re-circulated water in nurseries, with the implication that this could contribute to pathogen spread. In the UK, there also is a report indicating that *P. ramorum* was detected in irrigation ponds and that the contamination of the ponds was linked to infections on some landscape plantings.

Survival of propagules in container media (which almost never contain mineral soil) may differ from survival in mineral soils. Work with other *Phytophthora* species indicates that sporangia and zoospore cysts can survive for up to a month in soil, with survival times being shortest under dry conditions. Chlamydospores are formed initially in infected plant tissue, and are typically released as the tissue decomposes. They can survive, on average, six months or more, depending upon environmental conditions. However, survival data specific to *P. ramorum* is still being developed in several research laboratories.

Research needs: Controlled studies to clearly establish the role of contaminated water or potting soil in new plant infections; Experiments to determine the role of plant debris (infected, abscised leaves, etc.) in the epidemiology of *P. ramorum* within nurseries; Experiments to determine whether different container media components have differing influences on propagule survival; Experiments to determine parameters for efficacious pasteurization, sterilization, or composting of container media and/or components.

Question 7. Is there evidence that fungicide applications will prevent the development of symptoms, but allow survival of the pathogen in a host plant?

Response: The use of systemic fungicides has been shown to suppress symptoms for other *Phytophthora* species, and this also has been demonstrated for *P. ramorum*. Fungicide tests using different methods at four or more locations indicate that systemics such as Subdue Maxx are very effective in reducing leaf lesion size on several ornamental plants (rhododendrons, azaleas, camellia) and Christmas tree species. All materials tested were shown to be fungistatic, not fungicidal. In addition, there are a number of contact fungicides that have been shown to protect healthy plants from infection by *P. ramorum*. Since contact fungicides have no systemic activity, it is likely that they would not suppress symptom development on infected host tissues. It should be noted that suppression of *P. ramorum* sporulation by either systemic and/or contact materials has not yet been demonstrated.

It was clear during the public question period that growers feel trapped by rules that require them to withhold fungicide treatments from plants that might be placed on "hold." If the plants on hold are not infected, growers want to apply protection. If the plants on hold are infected, growers want to treat them so they cannot spread disease to other plants. Thus, from a risk management point of view, there is a strong rationale for using fungicides that can prevent new disease infections (i.e., contact protection of plant surfaces) as well as spread from existing infections (i.e., products that suppresses sporulation on lesions), while not masking symptom expression.

Needed research: We need a thorough comparison of systemic and contact fungicides for their relative ability to protect plant surfaces against infection, and their ability to suppress symptom development without killing the pathogen. If the materials suppress symptom development, we need to know how long suppression lasts. And if the materials allow survival of the pathogen, we need to know how long and in what form. Further, we need to know if sporulation is suppressed by any fungicides.

Question 8. What is the best, most reliable testing method for determining the presence or absence of the sudden oak disease (e.g. culture, nested PCR, etc.)?

Response: The best, most reliable, method is a combination of statistically valid sampling strategy and the use of a robust PCR method. ELISA should be a second choice screen because it is not sensitive enough, and the false negative and false positive rates are too high. With the recent completion of the genome sequence, the potential now exists to develop PCR assays with improved sensitivity and specificity. And there is a clear rationale for more than one approved test, so that samples can be cross-checked with another probe that detects another part of the genome. A shortcoming of the present, APHIS-approved PCR test is that it is known to cross react with *Phytophthora hibernalis*.

It was noted that PCR assays, in general, require a high standard of laboratory practice to perform properly. Thus, establishing uniform testing procedures is essential to the goal of accurate diagnosis and the ability to conduct independent verification of the same samples in different laboratories. Moreover, the panel concluded that whatever tests are used, they should be subjected to peer review before adoption as a national standard.

Research need: Development and validation of superior, and affordable, PCR tests based upon new gene sequences.

Question 9. Is there any specific data on the distance this pathogen could be expected to naturally migrate from plant to plant in a nursery situation, outside of a generally infested area?

Response: Although it hasn't been demonstrated for *P. ramorum,* there is literature relating to *P. palmivora*, which has a similar epidemiology, and which suggests a dispersal range of four meters by wind-blown rain.

Research need: Determine the role of airborne dispersal (i.e., free movement of sporangia on air currents) versus wind driven rain under nursery and natural situations.

Postscript: Throughout the public session, wherein responses were presented, the Panel noted that many questions cannot yet be answered definitively using data specific to *P. ramorum*. There is a significant amount of work in progress, and some research papers that have not yet appeared in print. Following is a listing of research projects under way, and manuscripts in press, to provide an overview of what is presently being done to answer these important questions. NOTE that this is not an exhaustive list, but rather a sampling to indicate the scope and nature of work under way.

Research Projects:

Following is a list of research projects recently funded by the UDSA Forest Service, Pacific Southwest Research Station, in cooperation with the California Department of Forestry and Fire Protection

BELTZ, H., BRAND, T., SEIPP, D., WAGNER, S., and WERRES, S. Chamber of Agriculture, Department of Horticulture, Bad Zwischenahn, Germany. Infectivity and survival of P. ramorum in recirculation water.

CUSHMAN, J. and MEENTEMEYER, R. California State University, Sonoma, CA. Influence of land-use history and vertebrates on the occurrence and spread of Phytophthora ramorum.

DOYLE, S. Joint Genome Institute, Department of Energy/University of California, Berkeley. Development of DNA aptamers for field detection of Phytophthora ramorum.

GOTTSCHALK, K., MacDONALD, W., JUZWIK, J., and LONG, R. USDA Forest Service, Northeastern Experiment Station, Morgantown, WV. Phytophthora ramorum in eastern United States forests: Sampling for presence and determining baseline Phytophthora species occurrence.

KELLY, M. University of California, Berkeley. Modeling potential spread of P. ramorum in the conterminous United States: effects of different models on modeled risk.

KELSEY, R. and MANTER, D. USDA Forest Service, Pacific Northwest Research Station, Corvallis, OR. Evaluating the role of host and non-host defensive chemicals on the pathogenicity and spore viability of Phytophthora ramorum.

MARTIN, F. USDA-Agriculture Research Service, Salinas, CA. Molecular diagnosis of Phytophthora spp., Sudden Oak Death as a case study.

MacDONALD, J., AND BOSTOCK, R. University of California, Davis, CA. The ecology and control of Phytophthora ramorum in nurseries.

PARKE, J. and LINDERMAN, R. Oregon State University and USDA-Agricultural Research Service, Corvallis, OR. Survival and dissemination of Phytophthora ramorum in soil and potting media.

STONE, J., and WINTON, L. Oregon State University, Corvallis, OR. Histopathology and PCR in situ visualization of Phytophthora ramorum within plant tissues.

SWIECKI, T. and BERNHARDT, E. Phytosphere Research Inc, Vacaville, CA. Key factors affecting disease risk, progression of disease and subsequent failure in trees infected with Phytophthora ramorum: A continuation of previous studies.

TJOSVOLD, S. University of California, Cooperative Extension, Watsonville, CA. Evaluation of fungicides for the control of Phytophthora ramorum infecting containerized Camellia, Viburnum and Pieris spp.

TJOSVOLD, S. University of California, Cooperative Extension, Watsonville, CA. The effect of soil inoculum concentration, presence of inoculum in irrigation water, irrigation method, and plant disease incidence on the epidemiology of Phytopthora ramorum affecting containerized Rhodendendron.

Research Papers:

Following is a list of research manuscripts submitted for publication, or recently accepted for publication, to illustrate some of the ongoing research that should, in time, help to answer questions about the biology and epidemiology of Phytophthora ramorum.

Dodd, Richard S., D. Hüberli, V. Douhovnikoff, T. Y. Harnik, Z. Afzal-Rafii and M. Garbelotto. 2004. Is variation in susceptibility to *Phytophthora ramorum* correlated with population genetic structure in coast live oak (*Quercus agrifolia*)? New Phytologist doi: 10.1111/j.1469-8137.2004.01200.x

Davidson, J. M., A. C. Wickland, H. A. Patterson, K. R. Falk, and D. M. Rizzo. Transmission of Phytophthora ramorum in mixed-evergreen forest in California. Phytopathology (in press).

Hayden, K. J., D. M. Rizzo, J. Tse and M. Garbelotto. 2004. Detection and quantification of Phytophthora ramorum from California forests using a real-time PCR assay. Phytopathology 94: 1075-1083.

Ivors K. L., K. J. Hayden, P. J. M. Bonants, D. M. Rizzo and M. Garbelotto. 2004. AFLP and phylogenetic analyses of North American and European populations of Phytophthora ramorum. Mycological Research 108: 378-392.

Kong, P., C.X. Hong, P.W. Tooley, K. Ivors, M. Garbelotto And P.A. Richardson. 2004. Rapid identification of Phytophthora ramorum using PCR-SSCP analysis of ribosomal DNA ITS-1. Letters in Applied Microbiology 38:433–439

Linderman, R. G., E. A. Davis, and J. L. Marlow. 2004/5. Comparative plant susceptibility and Phytophthora species' virulence on detached nursery crop leaves. Plant Disease (submitted)

Maloney, P. E., S. C. Lynch, S. F. Kane, C. E. Jensen, and D. M. Rizzo. Establishment of an emerging generalist pathogen in redwood forest communities. Journal of Ecology (in review).

Maloney, P. E., S. C. Lynch, S. F. Kane, and D. M. Rizzo. 2004. Disease progression of Phytophthora ramorum and Botryosphaeria dothidea on Pacific madrone. Plant Disease 88: 852-857.

Meentemeyer, R., D. M. Rizzo, R., W. Mark, and E. Lotz. 2004. Mapping the risk of establishment and spread of sudden oak death in California. Forest Ecology and Management 200: 194-215.

Tooley, P.W., Kyde, K.L. and Englander, L. 2004. Susceptibility of selected Ericaceous ornamental host species to *Phytophthora ramorum*. Plant Disease 88:993-999.